

Meta analysis of variant predictions in congenital adrenal hyperplasia caused by mutations in CYP21A2

Mayara J. Prado^{1,2,3,4}, Rodrigo Ligabue-Braun⁵, Arnaldo Zaha^{1,2}‡, Maria Lucia Rosa

Rossetti^{1,4}‡, Amit V. Pandey^{3,4} ‡

‡These authors contributed equally to this work.

¹Graduate Program in Cell and Molecular Biology, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre CEP 91501-970, Brazil. ² Center for Biotechnology, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre CEP 91501-970, RS, Brazil. ³ Department of Biomedical Research, University of Bern, Bern 3010, Switzerland. ⁴ Pediatric Endocrinology Unit, Department of Pediatrics, University Children's Hospital Bern, Bern 3010, Switzerland. ⁵ Department of Pharmacosciences, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre CEP 90050-170, Brazil. ⁶ Graduate Program in Molecular Biology Applied to Health, Universidade Luterana do Brasil (ULBRA), Canoas CEP 92425-020, Brazil.

ORCID numbers: M.J.P (0000-0003-0647-4429), R.L-B. (0000-0002-2555-9754), A.Z. (0000-0001-6336-474X), M.L.R.R (0000-0002-9672-9394), and A.V.P (0000-0001-8331-5902).

Table S1. Single predictors selected for performance analysis with *CYP21A2* variants.

Single Predictors	Description	Website	Ref.
CADD	Integrative annotation built based on diverse genomic feature derived from surrounding sequence context, gene model annotation , evolutionary constraint, epigenetic measurements, and functional predictions.	https://cadd.gs.washington.edu/	[1]
ConSurf	Algorithm uses phylogenetic relationships among homologous sequences and the specific dynamics of the analyzed sequence with evolutionary models to estimate the evolutionary rates of the amino acid of the macromolecules and to map them onto the structure and/or sequence.	https://consurf.tau.ac.il/	[2]
DANN	Deep neural network which takes non-linear relationships among features based on diverse genomic derived from surrounding sequence context, gene model annotation , evolutionary constraint, epigenetic measurements, and functional predictions.	https://cbcl.ics.uci.edu/public_data/DANN/	[3]
FATHMM	Evolutionary conservation algorithm which uses homologous sequences with species-specific weighting to predict the protein's tolerance to missense variants.	http://fathmm.biocompute.org.uk/	[4]
MAPP	A statistical framework predictor which uses protein physicochemical characteristics of each amino acid position on the evolutionary variation.	http://mendel.stanford.edu/sidowlab/downloads/MAPP/index.html	[5]
MutPred2	Machine learning-based to predict amino acid substitution through evolutionary , structural , and functional proprieties.	http://mutpred.mutdb.org/	[6]
PANTHER-PSEP	Predict using evolutionary preservation data, measuring though the length of time estimation that a site has been preserved.	http://www.pantherdb.org/tools/csnpscoreForm.jsp	[7]
PhD-SNP ^g	Machine learning algorithm for predicting SNVs in both non-coding and coding regions through evolutionary data .	https://snps.biofold.org/phd-snp/	[8]
PolyPhen-2	It uses human protein evolutionary and structural data to predict amino acid substitution effect on the protein stability and functionality.	http://genetics.bwh.harvard.edu/pph2/	[9]
PROVEN	Predict the functional effect through amino acid exchange evolutionary data and quality of the neighborhood sequence alignment rather than the target position.	http://provean.jcvi.org/genome_submit_2.php?species=human	[10]
SIFT	Predicts through sequence homology algorithm assuming evolutionary conserved regions tend to be less tolerant.	https://sift.bii.a-star.edu.sg/www/SIFT4G_vc_f_submit.html	[11]
SNAP2	A neural network method based on machine learning to predict the variant effect in the molecular function through evolutionary and structural protein data with an amino acid substitution matrix of effect probabilities.	https://roslab.org/services/snap2web/	[12]
SNPs&GO	Predict using evolutionary data, profile and gene ontology (biological process, cellular component and molecular function). When protein function is not available, it run PANTHER and PhD-SNP.	https://snps.biofold.org/snps-and-go/snps-and-go.html	[13]

18 Table S2. Performance of predictor tools.

Predictor	PPV	NPV	Se	Sp	Ac	MCC	AUC-ROC	Dataset	Ref.
CADD			93.6	57.1	0.85			ClinVar (2015)	[1]
DANN							0.95	ClinVar (2014)	[3]
FATHMM (weighted)	0.85	0.8	0.78	0.87	0.82	0.65		SwissVar (2012)	[4]
MAPP					0.626-0.767			Experimental studies	[5]
Meta-SNP	0.79	0.8	0.8	0.79	0.79	0.59	0.86	SwissVar (2009-2012)	[14]
MutPred2	96		42.3	95.6			84.9	ClinVar32 (2015) and UniProt80 (2015)	[6]
PANTHER-PSEP							0.721	Derived from SwissVar	[7]
PhD-SNPg	0.85	0.85	0.94	0.67	0.85	0.65	0.91	NewClinvar (2016)	[8]
PolyPhen-2*			0.85	0.6015			0.79	Mutations on the genes BRCA1, MSH2, MLH1 and TP53	[15]
PredictSNP					0.642	0.281	0.7	Protein Mutant Database (07Mar26)	[16]
PredictSNP2					0.773	0.55	0.804	Mendelian diseases (multiple databases)	[17]
PROVEAN			0.78	0.79				UniProt human protein	[10]
SNPs&GO	0.83	0.8	0.78	0.85	0.82	0.63		Derived from Swiss-Prot (2008)	[13]
SIFT 4G			0.8	0.735	0.7732	0.53		UniRef90 (2011)	[11]
SNAP2					0.688	0.24		Data set consisting of 9,657 variants from 678 human proteins	[12]
SNP&GO3d	0.84	0.86	0.87	0.83	0.85	0.7	0.92	Derived from Swiss-Prot (2009)	[18]

19 *Data from an article recommended on the original developer article. PPV, positive predictive value; NPV, negative predictive value; Se, sensibility; Sp, specificity; Ac, accuracy; MCC, Matthews'
20 correlation coefficient test.

Table S3..List of the 103 single nucleotide variants (SNVs) on *CYP21A2* gene selected to test the performance of predictor tools. SNVs are grouped into classical (enzyme activity < 10%), non-classical (between 10 and 78 %) and neutral (> 78 %) groups. The enzyme activity levels of both 21-hydroxylase substrates - 17-hydroxyprogesterone and progesterone - were obtained from the original paper of the functional characterization. The phenotype was obtained from either the same paper or the original description of the new SNV. ^a Shows the percentage of enzyme activity measured for the conversion of both 21-hydroxylase substrates, considering as 100 % the 21-hydroxylase wild type activity. 17OHP: 17-hydroxyprogesterone. SW: salt wasting. SV: simple-virilizing. NC: non-classical. ND: non-determinate.

Group	NP_000491.4	CYP21A2 Activity <i>in vitro</i>				Phenotype	Publication
		17OHP ^a	SD (±)	Progesterone	SD (±)		
CL	p.P31Q	0.2	0.2	0	0	SW	[19]
	p.G57R	0.7	ND	1.4	ND	SV	[20]
	p.G65E	0	ND	0	ND	SW	[21]
	p.I78T	3	2	5	3	SV	[22]
	p.G91V	0	ND	0	ND	SW	[23]
	p.L108R	0.4	ND	0.3	ND	SW	[20]
	p.S114F	4	1	4	2	SV	[24]
	p.L123P	1.42	2.13	-1.86	5.19	SW	[25]
	p.V140E	0.7	1.3	0.5	0.6	SW	[26]
	p.L143P	0.4		0.4		SW	[20]
	p.C148R	4.3	0.9	3.6ny	1.8	SV-NC	[26]
	p.L167P	0.3	0.06	0.4	0.6	SW	[27]
	p.L168P	0.7	ND	0.4	ND	SW	[28]
	p.C170R	0.1	0.02	0	2	SW	[29]
	p.I172N	0.7	0.3	0.6	0.03	SV	[30]
	p.I173N	4.3	1.7	4.4	1.8	SV	[28]
	p.G179R	0.4	0.5	0	0.6	SW	[29]
	p.R234G	8	2	2	1	SV-NC	[31]
	p.I237N	1	0.7	2.4	1.4	SV	[32]
	p.V238E	0	0	0.1	0.3	SW	[32]
	p.V282G	3.9	1.7	3.9	2	SV	[33]
	p.H283N	1.6	6	2.7	5	SW	[34]
	p.G292C	0	ND	0	ND	SW	[23]
	p.G292R	0.5	0.7	0.7	0.2	SW	[26]
	p.G292S	0.8	0.4	0.8	0.4	SW	[35]
	p.G293D	0.5	0.2	0.7	0.4	SW	[28]
	p.L301F	9.5	6.4	4.4	2.5	SV	[33]
	p.W303S	3	0.3	3	0.5	SV-NC	[36]
	p.W303R	0.1	0.2	0	0.5	SW	[29]
	p.L309F	0.2	0.3	0.1	0.3	SW	[26]
	p.E321K	4.6	1.8	4.5	2.6	SV	[28]
	p.R342P	0.7	0.3	0.7	0.2	SV	[30]
	p.R342W	5	0.4	4	3	SV-NC	[31]
	p.E352K	1.1	0.5	1.2	0.3	SV	[37]
	p.R355H	0	ND	0	ND	SW	[23]
	p.R357P	0.15	0.3	0.15	0.3	SW	[38]
	p.R357Q	0.65	0.44	1.1	0.94	SV	[38]
	p.R357W	0	ND	0	ND	SW	[39]
	p.A363V	0	ND	0	ND	SW	[21]
	p.G376S	1.6	0.8	0.7	0.7	SW	[40]
	p.L389R	1.1	0.6	ND	ND	SW	[41]

Continuation (Table S3)

CL	p.H393Q	2.5	0.6	2.2	0.6	SW	[42]
	p.R409C	1.3	0.5			SW	[20]
	p.G425S	1.6	0.4	2	0.6	SV	[28]
	p.R427C	0	0.5	0	0.6	SW	[29]
	p.R427H	0.5	0.6	0.4	0.2	SW-SV	[30]
	p.L447P	0.5	0.6	0	0.1	SW-SV	[30]
	p.T451P	0.9	ND	0.9	ND	SW	[24]
	p.P464L	2.6	0.8	3	0.5	SV	[43]
	p.R484P	1	0.07	2.2	0.9	SV	[35]
	p.R484Q	1.1	0.7	3.8	1.9	SV	[27]
Mean		1.52		1.32			
SD		2.00		1.61			
NC	p.P31L	13	0.2	2	0.6	NC	[31]
	p.H63L	44.5	ND	20.7	ND	NC	[20]
	p.P106L	62	9	64	12	NC	[44]
	p.H120R	31.6	8	32.5	7	NC	[45]
	p.K122Q	14	5	19.5	4	NC	[46]
	p.R133C	35.4	7.4	15.5	2.7	NC	[47]
	p.E141K	11.3	2.4	ND	ND	SW	[41]
	p.R150C	35.8	14.6	47.3	12.9	NC	[47]
	p.R150P	23.4	1.7	16.9	2	NC	[48]
	p.M151R	17.66	1.87	4.57	1.96	NC	[25]
	p.G179A	19	ND	ND	ND	NC	[23]
	p.Y192H	37.1	7	25.8	9	NC	[34]
	p.I195N	33.2	9	46.7	10	NC	[45]
	p.R225W	51.9	9	45.6	8	NC	[49]
	p.I231T	63.1	22.3	70.6	17	NC	[28]
	p.R234K	15	ND	8.1	ND	SV-NC	[28]
	p.V282L	18	3	18	5	NC	[31]
	p.M284V	16.2	9.3	19	6.8	NC	[47]
	p.V305M	46	18	26	10	NC	[50]
	p.F307V	63.23	5.5	64.17	7.98	SV-NC	[51]
	p.D323G	18	1.2	27	4.7	NC	[36]
	p.R340H	67.1	2.4	45.8	3.7	NC	[52]
	p.V359I	72	7	34	3	NC	[53]
	p.H366N	46.13	4.8	57.77	3.69	NC	[51]
	p.R367C	37	7	28	4	NC	[31]
	p.R370Q	82	6	63	4	NC	[53]
	p.R370W	45.8	1.8	48.5	17.1	NC	[28]
	p.D378Y	81	6	58	4	NC	[53]
	p.E381D	30	ND	ND	ND	SW	[54]
	p.A392T	38.7	9.5	22.9	4.7	NC	[55]
	p.D408N	72.7	7	73.6	10	NC	[49]
	p.E432K	26.2	3.8	24.2	7.4	NC	[47]
	p.A435V	14	2	12	6	SV	[22]
	p.T451M	78	6	43	5	NC	[24]
	p.P454S	38	ND	22.4	3	NC	[31]
	p.L462P	55	8	40	2	NC	[53]
	p.M474I	85	7	66	12	NC	[31]
	p.R480L	75.5	15.7	79.6	12	NC-Normal	[55]
	p.P483S	61	6	54	2	NC	[31]
Mean		42.94		37.41			
SD		22.59		20.98			

Continuation (Table S3)

Neutral	p.L13M	99	1	100	1	Normal	[24]
	p.A16T	100	0	96	6	Normal- very mildNC	[24]
	p.R17C	95	3	81	3	Normal- very mildNC	[24]
	p.R103K	119.7	22.5	ND	ND	Normal	[41]
	p.A160T	126.6	29.9	ND	ND	Normal	[41]
	p.D184E	100	ND	100	ND	Normal	[56]
	p.S203G	85	2	81	3	Very mild NC	[24]
	p.V212M	99.5	32.4	ND	ND	Normal	[41]
	p.M240K	95.4	24.7	97.7	7.7	Normal	[32]
	p.A266S	90	9	104	15	Normal	[31]
	p.A266V	92	1.4	100	4.3	Normal	[36]
	p.P268L	97	1	87	7	Normal	[24]
	p.S269T	103	15	ND	ND	Normal	[57]
	Average	100.17		94.08			
	SD	10.92		8.25			

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33 Table S4. Result of 17 predictors for 51 classical single nucleotide variants (SNVs) on the CYP21A2 gene. The classical group has an enzyme activity of < 10% of the
 34 wild-type activity. The genomic SNV nomenclature is based on the human chromatin remodeling 38 (Chr38). Del: deleterious; N: Neutral; Pby: Probably; Psb:
 35 Possible; B: Benign; Dse: Disease; Efc: Effect; P-Del: Proxy-deleterious; P-N: Proxy-neutral; Dmg: Damaging; T: Tolerated; Ptg: Pathogenic; Csv: Conserved; V:
 36 Variable; NR: no result.

Chr38	SNP	Meta-SNP	PredictSNP	PredictSNP2	S3Ds&GO	CADD	ConSurf	DANN	FATHMM	MAPP	MutPred2	PANTHER	PhD-SNPg	PolyPhen2	PROVEAN	SIFT	SNAP2	SNPs&GO
g.32038514C>A	p.P31Q	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	N;
g.32038591G>A	p.G57R	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32038616G>A	p.G65E	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32038752T>C	p.I78T	N	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	N	N	N	N
g.32038791G>T	p.G91V	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039124T>G	p.L108R	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039142C>T	p.S114F	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	B	Pby	Del	Del	Efc	Dse
g.32039169T>C	p.L123P	Dse	Del	N	Dse	P-Del	Csv	Del	T	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039220T>A	p.V140E	Dse	Del	N	Dse	P-Del	Csv	Del	T	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039229T>C	p.L143P	Dse	Del	N	Dse	P-Del	Csv	Del	T	Del	Del	Pby	Ptg	Pby	N	N	N	Dse
g.32039243T>C	p.C148R	N	N	N	N	P-Del	Csv	N	Dmg	Del	Del	Pby	Ptg	Psb	Del	N	N	N
g.32039408T>C	p.L167P	Dse	Del	N	Dse	P-Del	V	Del	T	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039411T>C	p.L168P	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	B	Pby	N	N	N	Dse
g.32039416T>C	p.C170R	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	B	Pby	Del	Del	Efc	Dse
g.32039423T>A	p.I172N	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039426T>A	p.I173N	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse

Continuation (Table S4)

Chr38	SNP	Meta-SNP	PredictSNP	PredictSNP2	S3Ds&GO	CADD	ConSurf	DANN	FATHMM	MAPP	MutPred2	PANTHER	PhD-SNPg	PolyPhen2	PROVEAN	SIFT	SNAP2	SNPs&GO
g.32039443G>A	p.G179R	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039797A>G	p.R234G	Dse	N	N	Dse	P-Del	Csv	Del	T	N	Del	Pby	B	Pby	Del	Del	Efc	Dse
g.32039807T>A	p.I237N	Dse	Del	N	N	P-Del	V	Del	T	Del	Del	Pby	Ptg	Psb	Del	Del	Efc	Dse
g.32039810T>A	p.V238E	Dse	Del	N	Dse	P-Del	Csv	Del	T	Del	Del	Pby	Ptg	Psb	Del	Del	Efc	Dse
g.32040111T>G	p.V282G	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040113C>A	p.H283N	Dse	N	N	Dse	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Pby	Del	Del	N	Dse
g.32040140G>A	p.G292S	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040140G>C	p.G292R	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040140G>T	p.G292C	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040144G>A	p.G293D	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040167C>T	p.L301F	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	N	Del	Pby	B	Pby	Del	Del	Efc	Dse
g.32040173T>C	p.W303R	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040174G>C	p.W303S	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040191C>T	p.L309F	N	N	Del	N	P-Del	Csv	Del	Dmg	N	N	Pby	B	Pby	Del	Del	N	N
g.32040427G>A	p.E321K	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040490C>T	p.R342W	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	N	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040491G>C	p.R342P	Dse	Del	N	Dse	P-Del	Csv	Del	T	Del	Del	Pby	B	Pby	Del	Del	Efc	Dse
g.32040520G>A	p.E352K	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040530G>A	p.R355H	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Psb	Del	Del	Efc	Dse

Continuation (Table S4)

Chr38	SNP	Meta-SNP	PredictSNP	PredictSNP2	S3Ds&GO	CADD	ConSurf	DANN	FATHMM	MAPP	MutPred2	PANTHER	PhD-SNPg	PolyPhen2	PROVEAN	SIFT	SNAP2	SNPs&GO
g.32040535C>T	p.R357W	Dse	Del	N	Dse	P-Del	Csv	Del	T	N	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040536G>A	p.R357Q	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040536G>C	p.R357P	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040554C>T	p.A363V	N	N	N	Dse	P-Del	Csv	Del	T	N	N	Pby	Ptg	Pby	N	N	N	N
g.32040675G>A	p.G376S	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	N	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040715T>G	p.L389R	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040728C>G	p.H393Q	N	N	N	Dse	P-Del	Csv	Del	T	N	Del	Pby	B	B	Del	Del	Efc	N
g.32040871C>T	p.R409C	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Psb	Del	Del	Efc	Dse
g.32040919G>A	p.G425S	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040925C>T	p.R427C	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040926G>A	p.R427H	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040986T>C	p.L447P	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	B	Pby	Del	Del	Efc	Dse
g.32040997A>C	p.T451P	Dse	Del	N	N	P-Del	Csv	Del	T	Del	Del	Pby	Ptg	Psb	Del	Del	N	N
g.32041037C>T	p.P464L	N	N	Del	N	P-Del	Csv	Del	Dmg	Del	N	Pby	Ptg	Pby	Del	Del	N	N
g.32041097G>A	p.R484Q	Dse	Del	Del	N	P-Del	Csv	Del	Dmg	N	Del	Pby	Ptg	Pby	Del	Del	Efc	N
g.32041097G>C	p.R484P	Dse	Del	N	N	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse

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39 Table S5. Result of 17 predictors for 39 non-classical single nucleotide variants (SNVs) on the CYP21A2. The non-classical group has an enzyme activity between
40 >10% and < 78% of the wild-type activity. The genomic SNV nomenclature is based on the human chromatin remodeling 38 (Chr38). Del: deleterious; N: Neutral;
41 Pby: Probably; Psb: Possible; B: Benign; Dse: Disease; Efc: Effect; P-Del: Proxy-deleterious; P-N: Proxy-neutral; Dmg: Damaging; T: Tolerated; Ptg: Pathogenic; Csv:
42 Conserved; V: Variable; NR: no result.

Chr38	SNP	Meta-SNP	PredictSNP	PredictSNP2	S3Ds&GO	CADD	ConSurf	DANN	FATHMM	MAPP	MutPred2	PANTHER	PhD-SNPg	PolyPhen2	PROVEAN	SIFT	SNP2	SNPs&GO
g.32038514C>T	p.P31L	N	N	N	N	P-Del	Csv	N	Dmg	Del	Del	Pby	Ptg	B	N	N	N	N
g.32038610A>T	p.H63L	Dse	N	N	N	P-Del	V	N	T	Del	Del	Pby	B	B	N	N	Efc	N
g.32039118C>T	p.P106L	N	N	N	N	P-N	V	N	T	N	N	Pby	B	B	N	N	Efc	N
g.32039160A>G	p.H120R	N	N	N	N	P-Del	Csv	Del	Dmg	Del	N	Pby	Ptg	Pby	Del	Del	Efc	N
g.32039165A>C	p.K122Q	Dse	Del	N	N	P-Del	Csv	Del	Dmg	Del	N	Pby	Ptg	Pby	Del	Del	Efc	N
g.32039198C>T	p.R133C	Dse	Del	N	Dse	P-Del	V	Del	T	N	N	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039222G>A	p.E141K	N	N	N	N	P-Del	V	Del	T	N	Del	Pby	Ptg	Psb	N	N	N	N
g.32039356C>T	p.R150C	N	N	Del	Dse	P-Del	Csv	Del	Dmg	N	N	Pby	B	Pby	N	N	N	N
g.32039357G>C	p.R150P	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	N	N	N	Dse
g.32039360T>G	p.M151R	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Psb	Del	Del	Efc	Dse
g.32039444G>C	p.G179A	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	N	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039570T>C	p.Y192H	N	N	N	N	P-N	Csv	N	T	N	N	Pby	B	B	N	N	N	N
g.32039580T>A	p.I195N	N	N	N	Dse	P-Del	Csv	Del	T	N	Del	Pby	Ptg	Pby	N	Del	Efc	Dse
g.32039770C>T	p.R225W	Dse	N	N	N	P-N	V	N	T	N	Del	Pby	B	B	Del	N	N	N
g.32039789T>C	p.I231T	N	N	N	N	P-Del	V	Del	T	N	N	Pby	B	B	N	Del	N	N
g.32039798G>A	p.R234K	N	N	N	Dse	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Pby	N	Del	Efc	N

Continuation (Table S5)

Chr38	SNP	Meta-SNP	PredictSNP	PredictSNP2	S3Ds&GO	CADD	ConSurf	DANN	FATHMM	MAPP	MutPred2	PANTHER	PhD-SNPg	PolyPhen2	PROVEAN	SIFT	SNP2	SNPs&GO
g.32040110G>T	p.V282L	N	N	N	N	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Psb	N	N	N	N
g.32040116A>G	p.M284V	N	N	Del	Dse	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040179G>A	p.V305M	Dse	N	Del	N	P-Del	Csv	Del	Dmg	N	N	Pby	B	Pby	N	Del	N	N
g.32040185T>G	p.F307V	N	N	N	Dse	P-Del	Csv	Del	Dmg	N	Del	Pby	B	Pby	Del	Del	N	Dse
g.32040434A>G	p.D323G	Dse	Del	N	N	P-Del	V	Del	T	Del	Del	Pby	B	Pby	Del	Del	N	N
g.32040485G>A	p.R340H	Dse	Del	Del	N	P-Del	Csv	Del	Dmg	N	Del	Pby	B	Pby	Del	Del	Efc	Dse
g.32040541G>A	p.V359I	N	N	N	N	P-Del	Csv	Del	Dmg	N	N	Pby	B	Psb	N	Del	N	N
g.32040562C>A	p.H366N	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	N
g.32040565C>T	p.R367C	N	N	N	N	P-Del	Csv	Del	T	N	Del	Pby	B	B	N	N	N	N
g.32040574C>T	p.R370W	Dse	Del	N	Dse	P-Del	Csv	Del	T	Del	N	Pby	B	Pby	Del	Del	Efc	Dse
g.32040575G>A	p.R370Q	N	N	N	N	P-Del	Csv	Del	T	N	N	Pby	B	Psb	N	N	N	N
g.32040681G>T	p.D378Y	N	N	N	N	P-Del	Csv	Del	T	N	Del	Pby	B	Psb	Del	N	N	N
g.32040692G>C	p.E381D	N	N	Del	N	P-Del	Csv	Del	Dmg	N	N	Pby	B	B	N	Del	Efc	N
g.32040723G>A	p.A392T	N	N	Del	Dse	P-Del	Csv	Del	Dmg	Del	N	Pby	B	Pby	N	N	N	Dse
g.32040771G>A	p.D408N	N	N	Del	N	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Pby	N	Del	N	N
g.32040940G>A	p.E432K	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	N	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040950C>T	p.A435V	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040998C>T	p.T451M	N	Del	N	N	P-Del	Csv	Del	T	Del	N	Pby	Ptg	Psb	Del	Del	N	N
g.32041006C>T	p.P454S	N	Del	Del	N	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Pby	Del	Del	N	N

Continuation (Table S5)

Chr38	SNP	Meta-SNP	PredictSNP	PredictSNP2	S3Ds&GO	CADD	ConSurf	DANN	FATHMM	MAPP	MutPred2	PANTHER	PhD-SNPg	PolyPhen2	PROVEAN	SIFT	SNP2	SNPs&GO
g.32041031T>C	p.L462P	Dse	Del	Del	N	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	N	N
g.32041068G>T	p.M474I	N	N	N	N	P-N	V	N	T	N	N	Pby	B	B	Del	N	N	N
g.32041085G>T	p.R480L	N	N	N	N	P-Del	V	Del	T	N	N	Pby	B	B	N	N	N	N
g.32041093C>T	p.P483S	N	N	Del	N	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Pby	N	Del	Efc	N

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45 Table S6. Result of 17 predictors for 13 neutral single nucleotide variants (SNVs) on the CYP21A2 gene. The neutral group has the enzyme activity known as > 78%
 46 of the wild-type activity. The genomic SNV nomenclature is based on the human chromatin remodeling 38 (Chr38). Del: deleterious; N: Neutral; Pby: Probably;
 47 Pbs: Possible; Dse: Disease; Efc: Effect; P-Del: Proxy-deleterious; P-N: Proxy-neutral; Dmg: Damaging; T: Tolerated; Ptg: Pathogenic; B: Benign; Csv: Conserved; V:
 48 Variable; NR: no result.

Chr38	SNP	Meta-SNP	PredictSNP	PredictSNP2	S3Ds&GO	CADD	ConSurf	DANN	FATHMM	MAPP	MutPred2	PANTHER	PhD-SNPg	PolyPhen2	PROVEAN	SIFT	SNP2	SNPs&GO
g.32038459C>A	p.L13M	N	N	N	NR	P-Del	NR	Del	T	Del	N	NR	B	Pby	N	Del	N	N
g.32038468G>A	p.A16T	N	N	N	NR	P-N	NR	N	T	N	N	NR	B	B	N	N	N	N
g.32038471C>T	p.R17C	N	N	N	NR	P-N	NR	Del	T	N	N	NR	B	B	N	Del	N	N
g.32039109G>A	p.R103K	N	N	N	N	P-N	V	N	T	N	N	NR	B	B	N	N	N	N
g.32039386G>A	p.A160T	N	N	N	N	P-N	Csv	N	T	N	N	Pby	B	B	N	N	N	N
g.32039548C>G	p.D184E	N	N	N	N	P-N	V	N	T	N	N	Pby	B	B	N	N	N	N
g.32039603A>G	p.S203G	N	N	N	N	P-N	V	N	T	N	N	Pby	B	B	N	N	N	N
g.32039630G>A	p.V212M	N	N	N	N	P-N	V	Del	T	N	N	Pby	B	B	N	N	N	N
g.32039816T>A	p.M240K	N	N	N	N	P-Del	V	N	T	N	N	Pby	Ptg	B	N	N	N	N
g.32040062G>T	p.A266S	N	N	N	N	P-N	V	N	T	N	N	Pby	B	B	N	N	N	N
g.32040063C>T	p.A266V	N	N	N	N	P-N	V	Del	T	Del	N	Pby	B	B	N	N	N	N
g.32040069C>T	p.P268L	N	N	N	NR	P-N	V	N	T	N	N	Pby	B	B	N	N	N	N
g.32040072G>C	p.S269T	N	N	N	NR	P-N	V	N	T	NR	N	Pby	B	B	N	N	N	N

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